Size and Charge Characteristics of the Protein Leak in Dengue Shock Syndrome


Background. The mechanism underlying the transient vascular leak syndrome of dengue hemorrhagic fever (DHF) is unknown. We aimed to determine whether molecular size and charge selectivity, which help restrict plasma proteins within the intravascular space, are altered in patients with DHF and whether a disturbance of the anionic glycosaminoglycan (GAG) layer on the luminal endothelial surface contributes to disease pathogenesis.

Methods. We measured serial plasma levels and fractional clearances of proteins with different size and charge characteristics in 48 children with dengue shock syndrome (DSS) and urinary excretion profiles of heparan sulfate, chondroitin-4-sulfate, and chondroitin-6-sulfate in affected children and healthy control subjects.

Results. Compared with convalescent values, acute plasma concentrations of all proteins were reduced, with increased fractional clearances. Smaller proteins were more affected than larger molecules. Albumin, which is normally protected from leakage by its strong negative charge, demonstrated a clearance pattern similar to that of transferrin, a neutral molecule of similar size. Urinary heparan sulfate excretion was significantly increased in children with DSS.

Conclusions. The endothelial size-dependent sieving mechanism for plasma proteins is at least partially retained, whereas selective restriction based on negative charge is impaired. The increased heparan sulfate excretion suggests a role for GAGs in the pathogenesis of the vascular leak.

The latter half of the 20th century has seen the emergence of dengue as one of the principal infectious diseases of the tropical world [1]. At present, some 2.5 billion people live in areas of risk, between 250 and 500,000 cases of dengue hemorrhagic fever (DHF) are reported to the World Health Organization (WHO) annually, and an estimated 100 million cases of dengue fever (DF) occur worldwide each year [2, 3]. During a recent epidemic in 1998, almost 120,000 cases of DHF and 342 deaths were reported in southern Vietnam alone, most of which occurred in children [4].

The cardinal feature that is thought to distinguish DHF from DF is a transient increase in vascular permeability that results in the leakage of fluid from the plasma to the interstitium [5]. The circulating blood volume is reduced, hemoconcentration occurs, and, in severe cases, hypovolemic shock develops. After a few days, the increase in permeability resolves spontaneously, the leaked fluid is reabsorbed, and the patient recovers quickly. Treatment is supportive and primarily relies on judicious volume replacement [6]. The pathophysiological mechanisms underlying the capillary leak are poorly understood. At present, there is no practical means of predicting which of the many dengue-infected patients are likely to develop this potentially life-threatening complication. Many factors—including total virus burden, viral virulence, host immune response, and genetic predisposition—have been implicated in the pathogenesis of DHF, but the means by which any of these factors might influence endothelial function remain unclear [7–10].

In the extracellular fluid compartment, plasma and
interstitial fluid exist in dynamic equilibrium separated by the semipermeable capillary wall [11]. The transport of water and macromolecules across the microvascular endothelium is largely passive and is governed by the net Starling forces (i.e., the balance between opposing hydrostatic and colloid osmotic pressures in the microcirculation) [12]. The relative excess of protein in plasma, compared with interstitial fluid, is critical in maintaining this balance. Molecular size, configuration, and charge all play a role in determining the disposition and movement of macromolecules within the extracellular system [13, 14]. Very small solutes are freely filtered, whereas the clearance of larger molecules decreases with increasing size, and those with a molecular radius of more than ~42 Å are almost entirely restricted within plasma [15]. Albumin (molecular radius, ~36 Å), the major protein responsible for the colloidal properties of plasma, carries a strong negative charge and is filtered less readily than neutral proteins of similar size, such as transferrin [16, 17].

Glycosaminoglycans (GAGs) are complex, negatively charged polysaccharides that are widely distributed on cell surfaces and are incorporated into the glycocalyx layer on the luminal surface of the vascular endothelium [18, 19]. The detailed structure of the glycocalyx is not yet established, but it is thought to consist of an ordered matrix of fibers and adherent plasma proteins, anchored in the underlying endothelial cells and extending throughout all vascular beds. It creates both a size-selective physical barrier that only allows the passage of certain molecules between the fibers and an electrostatic barrier that limits the access of negatively charged molecules to the underlying cellular transport mechanisms [13, 20]. Disruption of the GAG components of the glycocalyx layer has been implicated in the increased clearance of proteins seen in animal models of capillary leak [21, 22] and in the pathogenesis of renal protein-losing disorders, such as steroid responsive-nephrotic syndrome (SRNS), and generalized vascular leak syndromes, such as meningococcal septicemia [23–25].

The investigation of the mechanisms of systemic capillary leak in vivo is complicated by the difficulties of obtaining interstitial fluid for examination. Although there are differences in endothelial cell architecture in capillaries of different organs, the basic Starling forces that control microvascular permeability are similar in all vascular beds, and it is the characteristics of the ubiquitous glycocalyx layer that are the major determinants of perselectivity, rather than those of the underlying cellular structures [13, 26]. Protective adaptations in the glomerular capillaries, which are intended to preserve the intravascular albumin pool in the face of a glomerular filtration rate of the order of 180 L/day in adults, result in the production of virtually protein-free urine in normal circumstances—that is, the threshold for protein loss from the glomerular capillaries to Bowman’s space is higher than that from systemic capillaries to the interstitium [27]. Therefore, increases in glomerular protein permeability in patients without renal disease are likely to indicate substantial increases in systemic permeability. The urinary clearance of proteins must be related to the clearance of a reference marker, to adjust for the glomerular filtration rate. This also allows clearance calculations to be based on simultaneous blood and urine samples rather than cumbersome and, frequently, inaccurately timed urine collections. Fractional clearance methodology, using creatinine as the reference marker, is well established for investigating renal disorders in pediatric practice [28] and has also provided useful insights into the pathogenesis of the systemic leak associated with meningococcal septicemia [25].

Although the clinical features of DHF suggest that vascular endothelial dysfunction is a prominent feature of the disease and alterations in microvascular permeability have been demonstrated using strain gauge plethysmography [29], there is no evidence that the virus directly infects endothelial cells in vivo, and no structural endothelial abnormalities have been demonstrated [30]. Hypoalbuminemia and proteinuria have been reported [31], but no other information is currently available with regard to the nature of the capillary leak, the characteristics of the proteins involved, or the severity and time course of the pathological process. We therefore decided to investigate the nature of the capillary leak in children with dengue shock syndrome (DSS), the most severe manifestation of DHF, by measuring serial plasma levels and fractional urinary clearances of selected plasma proteins with different size and charge characteristics. Also, we recently noted significant reductions in the plasma levels of the natural anticoagulant proteins antithrombin, protein C, and protein S in children with acute DSS but without comparable abnormalities in the standard coagulation screening tests [32]. Because this pattern would be more consistent with leakage of these proteins from the plasma than with consumption, we decided to include 1 representative anticoagulant protein, antithrombin, in the present study. Finally, although the GAG heparan sulfate is an important cellular receptor for dengue viral adhesion, no research has been done to investigate whether the disruption of the GAG component of the glycocalyx layer might be a feature of the capillary leak syndrome in DHF [33, 34]. We therefore measured the urinary excretion profiles of the 3 major GAGs (heparan sulfate, chondroitin-6-sulfate, and chondroitin-4-sulfate) that are important in maintaining the perselective properties of the capillary wall in the same group of children and compared the results with those from healthy control subjects.

PATIENTS, MATERIALS, AND METHODS

Patients and clinical methods. Since 1998, all children with a clinical diagnosis of DSS (table 1) admitted to the pediatric intensive care unit at the Hospital for Tropical Diseases of Ho Chi Minh City, Vietnam, have been enrolled into an ongoing series of clinical trials focused on fluid resuscitation, provided
that informed consent is given by a parent or guardian. Both clinical and laboratory aspects of the studies have been approved by the Ethical Committee of the Hospital for Tropical Diseases of Ho Chi Minh City. Information on criteria for enrollment, general and fluid management, monitoring, and severity scoring has been detailed elsewhere [32]. In brief, all patients are managed in a standardized manner after an initial bolus of randomized and blinded resuscitation fluid, which may be either lactated Ringers’ solution or 1 of 2 colloids (6% dex-

tran 70 or 6% hydroxyethyl starch). Simultaneous plasma and urine samples are obtained as follows: at presentation, before resuscitation (day 1), the following morning (day 2), at discharge (for serological testing), and at a 1-month follow-up visit (from all survivors who attend). Plasma samples are separated as soon as possible and are stored at −70°C; urine samples are stored at −30°C. After discharge, the severity of shock is classified as mild, moderate, or severe, according to the time taken to achieve cardiovascular stability (defined as a sustained pulse pressure of ≥25 mm Hg with a systolic pressure appropriate for age) and the requirement for additional rescue colloid.

For the present study, we chose a group of 48 patients who were admitted with DSS during the 1998–1999 dengue season. All of those classified with severe shock during the season were included, together with a selection of those classified as having mild and moderate shock, who were chosen for the completeness of the acute and convalescent sample sets. The chosen study group has been shown elsewhere to be representative of the whole group of children admitted with DSS during that season [32]. For the protein clearance studies, individual patients acted as their own controls, with the follow-up results considered to be normal. For the GAG studies, healthy schoolchildren 6–8 years old provided control urine samples, but we did not consider it ethical to request blood samples from these children. Thus, the GAG data are presented as urinary excretion ratios, expressed relative to creatinine excretion, rather than as fractional clearances.

**Laboratory methods.** The frozen samples were transported on dry ice to St. Mary’s Hospital, London, for analysis. Dengue is classified as a category 3 pathogen in the United Kingdom, so all samples were inactivated with the virucidal agent Triton X-100 (0.5% final concentration) before handling [35]. Although Triton may interfere with certain laboratory assays, preliminary studies comparing treated and untreated aliquots of plasma and urine from volunteers showed that there was no effect on the various assays of interest for the present study (data not shown).

Dengue infection was confirmed serologically using the dengue Duo IgM and IgG Capture ELISA kit (Panbio). Plasma albumin (bromocresol green method) and creatinine (alkaline picrate method) were measured by use of an Olympus AU 640 analyzer (Olympus Diagnostica), and transferrin and IgG were measured by rate nephelometry on a Beckman Immage analyzer (Beckman Coulter) in the Department of Chemical Pathology, St. Mary’s Hospital. Plasma antithrombin concentrations for this group of children have been reported elsewhere [32]. Urine concentrations of albumin, transferrin, IgG, and antithrombin were measured by ELISA, as described elsewhere, in each case by use of the appropriate rabbit antihuman antibodies (Dako) [36]. Urine creatinine concentrations were measured by use of the Jaffe method. Fractional urinary clearances for the various proteins were calculated relative to that of creatinine as follows:

\[
\text{Fractional clearance of protein} = \frac{(U_{\text{protein}}/V)(P_{\text{protein}})}{(U_{\text{creatinine}}/V)(P_{\text{creatinine}})} \times 100
\]

where \(V\) is the volume of urine (mL), \(U_{\text{protein}}\) is the urine protein concentration (g/L), \(P_{\text{protein}}\) is the plasma protein concentration (g/L), \(U_{\text{creatinine}}\) is the urine creatinine concentration (μmol/L), and \(P_{\text{creatinine}}\) is the plasma creatinine concentration (μmol/L).

Chondroitin-6-sulfate, chondroitin-4-sulfate, and heparan sulfate were precipitated from urine and separated electrophoretically according to the method of Wessler and Whiteman, with minor modifications [25, 37, 38]. The GAG analyses were performed on urine samples only; without contemporaneous blood

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**Table 1. World Health Organization guidelines for the clinical diagnosis of dengue shock syndrome in endemic areas [6].**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Notes</th>
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<tbody>
<tr>
<td>Fever</td>
<td>2–7 days</td>
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<tr>
<td>Hemorrhagic tendency</td>
<td>Any of the following: positive tourniquet test result; spontaneous petechiae or other skin bleeding; or mucosal or gastrointestinal tract bleeding</td>
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<tr>
<td>Thrombocytopenia</td>
<td>Platelet count ≤100,000/mm³</td>
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<tr>
<td>Evidence of plasma leakage</td>
<td>Any of the following: increase in admission hematocrit to &gt;20% above the expected mean for age, sex, and population; decrease in hematocrit to &gt;20% of the baseline value after resuscitation; or clinical signs of plasma leakage, such as pleural effusion or ascites</td>
</tr>
<tr>
<td>Circulatory compromise</td>
<td>Narrow pulse pressure ≤20 mm Hg, with tachycardia, or hypotension for age</td>
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</tbody>
</table>

* A few patients, although thrombocytopenic, did not have absolute platelet counts ≤100,000 mm³ at the time of admission.
results, fractional clearances could not be calculated for these data. The results are presented with the concentration of GAG in each urine sample expressed as a ratio of urine creatinine, to allow for differences in the state of hydration and urinary concentrating ability between individuals.

**Statistical analysis.** All results have been summarized in terms of median and range. Comparisons between results obtained from the same child at different time points were performed by use of the Wilcoxon signed-rank test for continuous variables. Comparisons between patient groups were done by use of the nonparametric Kruskal-Wallis or Mann-Whitney U test. The Spearman rank correlation coefficient was used to examine the relationships between different variables. All statistical computations were done by use of the program SPSS (version 10 for Windows; SPSS).

**RESULTS**

Admission characteristics and basic laboratory investigations for the 48 children, all of whom were confirmed to have secondary dengue infection, are reported in table 2. Four of the children died from severe shock that was unresponsive to aggressive management (median [90% range] rescue colloid volume, 47 [32–69] mL/kg before death), 16 had moderately severe shock (median [90% range] rescue colloid volume, 15 [10–33] mL/kg; median [90% range] shock recovery time, 20 [12–38] h), and the remaining 28 children had mild shock, stabilizing without the need for rescue colloid after a median (90% range) shock recovery time of 1.5 (1–6) h. The moderate and severe shock groups were combined for the purposes of the present analysis. The mild and moderate/severe groups were comparable, apart from minor but significant differences in admission pulse and blood pressure (table 2). Urine output was not reduced in the majority of children, except during the final terminal stages in the 4 who died. In none of the other children was renal dysfunction apparent. Acute plasma creatinine concentrations were not significantly different from the convalescent values: median (90% range): day 1, 68 (46–117); day 2, 60 (43–88); and 1 month, 63 (51–83) µmol/L. A small number of children were treated for fluid overload with a diuretic but always later during the course of the illness, after all acute samples had been obtained.

Plasma concentrations of IgG, transferrin, and albumin were significantly reduced ($P<.001$ for all comparisons) at presentation with DSS, compared with convalescent values (figure 1). Convalescent concentrations of all 3 proteins were within the normal range. The median (90% range) plasma albumin concentration for the whole group before resuscitation was 27 (17–33) g/L. At this time, all children exhibited marked hemoconcentration (median [90% range] hematocrit, 49% [42%–57%]). This degree of hemoconcentration would be expected to increase the concentrations of all plasma constituents; thus, the true albumin concentrations must have been considerably lower than these measured values. The concentrations of all 3 proteins decreased to lower levels on shock day 2; by this time, the children had received considerable parenteral fluid therapy, although many still had some degree of hemoconcentration, which indicates ongoing volume depletion. In children with moderate/severe shock, the protein levels were significantly lower than in patients with mild shock ($P<.01$ for all comparisons). Considering the convalescent values to be represen-

### Table 2. Admission characteristics of 48 children with dengue shock syndrome, presented according to severity of shock as assessed at discharge or death.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mild (n = 28)</th>
<th>Moderate/severe (n = 20)</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (90% range), years</td>
<td>9 (4–14)</td>
<td>8 (3–13)</td>
<td>NS</td>
</tr>
<tr>
<td>Male sex, no. (%)</td>
<td>12 (43)</td>
<td>9 (45)</td>
<td>NS</td>
</tr>
<tr>
<td>Day of illness when presenting with shock, median (90% range)</td>
<td>5 (3–8)</td>
<td>5 (3–10)</td>
<td>NS</td>
</tr>
<tr>
<td>Unrecordable pulse, no. (%)</td>
<td>2 (7)</td>
<td>2 (10)</td>
<td>NS (F)</td>
</tr>
<tr>
<td>Pulse rate, median (90% range), beats/min$^b$</td>
<td>110 (88–130)</td>
<td>120 (110–140)</td>
<td>.001</td>
</tr>
<tr>
<td>Unrecordable blood pressure, no. (%)</td>
<td>1 (4)</td>
<td>4 (20)</td>
<td>NS (F)</td>
</tr>
<tr>
<td>Systolic blood pressure, median (90% range), mm Hg$^b$</td>
<td>100 (80–120)</td>
<td>90 (80–100)</td>
<td>.01</td>
</tr>
<tr>
<td>Admission pulse pressure, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\leq$10 mm Hg</td>
<td>10 (36)</td>
<td>6 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>$&gt;10\leq20$ mm Hg</td>
<td>18 (64)</td>
<td>14 (70)</td>
<td>NS</td>
</tr>
<tr>
<td>Spontaneous bleeding, no. (%)</td>
<td>24 (86)</td>
<td>15 (75)</td>
<td>NS (F)</td>
</tr>
<tr>
<td>Hematocrit, median (90% range), %</td>
<td>49 (42–57)</td>
<td>50 (40–57)</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count, median, (90% range), cells/mm$^3$</td>
<td>98,000 (15,000–150,000)</td>
<td>70,000 (9000–180,000)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** NS, not significant.

$^a$ For categorical variables, $\chi^2$ test or Fisher’s exact test (F). For continuous variables, nonparametric Mann-Whitney U test.

$^b$ For those with recordable values.
Figure 1. Serial plasma levels and fractional urinary clearances for IgG, transferrin, and albumin in children with acute dengue shock syndrome, by day of shock, where day 1 is the day of presentation with shock. Comparisons for days 1 and 2 blood \((n = 38\) and 39\) and urine \((n = 27\) and 34\) results for the combined severity groups with convalescent values were all highly significant \((P < .001, \text{ Wilcoxon signed-rank test for paired data})\). Within-day comparisons between the mild shock and moderate/severe shock groups were significant for all 3 plasma protein concentrations \((P < .01, \text{ Mann-Whitney } \text{ } U \text{ test})\) but not for the urinary clearances. Dotted arrows, approximate ranges seen in acute meningococcal sepsis [25].

Tative of baseline concentrations before illness, acute plasma levels of the smaller proteins were decreased significantly more than those of IgG—median (90% range) reductions for albumin (molecular weight \([\text{MW}]\), 69,000 Da) and transferrin \((\text{MW}, 79,000 \text{ Da})\) were 36% (6%–56%) and 34% (9%–53%), respectively, on day 1, whereas that for IgG \((\text{MW}, 150,000 \text{ Da})\) was 20% (−12%–52%) \((P < .001 \text{ for albumin and transferrin vs. IgG but no significant difference for albumin vs. transferrin})\). In a few patients, IgG levels were actually higher at admission, which probably reflects the intravascular volume depletion.

Acute fractional urinary clearances of all proteins were increased significantly, compared with convalescent values \((P < .001 \text{ for all comparisons})\), but those of transferrin and albumin were more affected than IgG clearance (figure 1). Control data were not available, because of the decision not to obtain blood samples from healthy children, but the convalescent clearances for the proteins were similar to those seen in a small number of healthy European children in a study of meningococcal sepsis [25]. No statistical differences were observed in the urinary clearances between the shock severity groups. Considering all
results on shock day 2 (n = 41), there was a strong correlation between the fractional urinary clearance of albumin and that of transferrin (Spearman correlation, 0.9; P < .001) and a weaker, although still highly significant, relationship between albumin and IgG clearances (Spearman correlation, 0.6; P < .001). The fractional clearance of antithrombin, a molecule with a MW of 59,000 Da and only a mild negative charge, demonstrated the same pattern of markedly increased excretion during acute DSS as albumin and transferrin, and the correlation with albumin clearance was strong (Spearman correlation, 0.8; P < .001) (figure 2).

Data for the urinary excretion of the 3 GAGs are presented in table 3. Urinary heparan sulfate and creatinine excretion was significantly greater in the children with acute DSS, compared with that in healthy control subjects of similar age (P = .016). No significant differences were observed in the excretion patterns of the other GAGs.

**DISCUSSION**

We have shown that concentrations of a series of proteins of different molecular size are all markedly reduced in the plasma of children presenting with acute DSS and that this reduction correlates with clinical severity. We have also demonstrated a corresponding increase in fractional urinary clearances of the same proteins and have confirmed that leakage contributes significantly to the reduction in plasma concentrations of the anticoagulant protein antithrombin. The smaller proteins (MW, 59,000–79,000 Da) were more affected than IgG (MW, 150,000 Da), and there were strong correlations between proteins of similar size, which suggests that the usual size-dependent sieving mechanism is at least partially retained. Clearances of albumin, which is usually protected from leakage by its strong negative charge, and transferrin, a neutral molecule, correlated closely with each other, which suggests that the selective restriction based on negative charge may be impaired. These results point toward an alteration in the function of the endothelial glycocalyx during dengue infection.

The use of urine as a fluid to study the mechanisms of vascular leak can be criticized for several reasons. First, the tubular reabsorption of proteins filtered in the glomerulus may significantly reduce the quantities measured in urine, although, at high rates of protein clearance, the tubular capacity for reabsorption is exceeded [39, 40]. However, any error introduced in this way would have resulted in falsely low, rather than increased, clearances of the various proteins. Second, the impairment of renal function may influence protein clearance. All of the children fulfilled the WHO cardiovascular criteria for DSS [6]—most were in a state of early or “compensated” shock at admission. Once resuscitation was initiated, good urine flows were established quickly in most cases, with little change in plasma creatinine levels over time. Any confounding effects are likely to have been minimal, and we consider the changes in protein clearance that we have demonstrated to be representative of the increased permeability of the glomerular capillary wall to macromolecules and to reflect the pathophysiological process occurring in systemic capillary beds.

The levels of hypoproteinemia were profound, and the fractional protein clearances observed were similar in magnitude
to those seen in meningococcal sepsis (figure 1), the only other disease in which hypovolemic shock secondary to vascular leak commonly occurs [25]. Other rare causes of shock in children include major trauma and hemorrhage, but, in these situations, a loss of whole blood is the primary reason for the cardiovascular collapse, rather than vascular leak. Although no information is available with regard to fractional protein clearances in traumatic or hemorrhagic shock, it seems likely that the results reported here and for meningococcal sepsis are indicative of the underlying vascular leak process rather than a consequence of hypovolemia.

In meningococcal sepsis, the excretion of all 3 GAGs is substantially increased, probably as a result of the cleavage of the glycoproteins from the endothelial surface and release into the blood and urine [41]. However, the pattern of GAG excretion seen in our patients was more similar to that found in SRNS, with a small but significant increase demonstrated only in the urinary heparan sulfate:creatinine ratio [25]. In SRNS, there is evidence that the negative charge of the capillary wall is neutralized by an adherent cationic protein rather than by cleavage of GAGs [24, 42]. It is possible that a comparable mechanism operates in children with DSS. The dengue virus is known to adhere to heparan sulfate [33, 34], and maximum plasma viremia levels have been shown to correlate with the degree of plasma leakage in some, although not all, studies [7, 43, 44]. Recently, dengue viral RNA has been demonstrated to persist in immune complexes during defervescence, which is the time when vascular leak usually becomes apparent [45]. Possible interactions among dengue viruses, immune complexes, and other components of the immune response with endothelial heparan sulfate and other GAGs merit further investigation.

In addition to shedding light on the possible mechanism of the protein leak in DHF, the present data encourage a reevaluation of dengue pathophysiology in general. Conventionally, the onset of capillary leak in DHF is said to occur at about the time of defervescence, because hemoconcentration usually becomes apparent at this time [6]. However, the normal regulation of plasma volume within tightly circumscribed limits is a complex process, and a substantial reserve exists to buffer the effects of any disturbances [11]. Lymphatic flow increases substantially to compensate for increases in ultrafiltration, and hypovolemia and hemoconcentration are generally late manifestations of disturbances in the plasma volume equilibrium and indicate that the normal compensatory mechanisms have been exhausted [46, 47]. Although catastrophic leak occurring over a short period of time, as in meningococcal septicemia or anaphylactic shock, may overwhelm these mechanisms, such severe leak is invariably accompanied by acute cardiovascular collapse. As we have demonstrated in the present study, all of our patients with DSS manifested significant hypoproteinemia that was often equivalent to a loss of more than one-third of their intravascular protein mass, yet many of them exhibited only minor degrees of cardiovascular compromise. A relatively slow protein leak that allows time for the activation of homeostatic regulatory mechanisms seems more in keeping with these clinical observations than a sudden, massive leak. Only in those patients in whom the leak exceeds the regulatory capacity is hemoconcentration, later followed by cardiovascular compromise, likely to become apparent. One possible implication of this is that patients with DF may also have mild capillary leak that occurs at a rate that is insufficient to result in hemoconcentration or a discernible increase in interstitial fluid volume and is therefore clinically undetectable. The hypothesis that quantitative differences in protein leak explain the apparent demarcation between DF and DHF, rather than a discrete pathological process that occurs only in individuals with DHF, is the subject of further work by our group.

The recognition that urinary protein leak can provide useful information about systemic capillary function, together with the availability of reliable techniques to document small changes in protein clearance, presents a novel and interesting approach to try to unravel the complexities of dengue pathophysiology and thereby improve case management. The early onset and/or severity of urinary protein leak may be useful predictors for the subsequent development of DHF and DSS, and, in due course, simple tests of urine protein excretion might prove to be effective for monitoring the many thousands of children with suspected dengue infection who present to health-care facilities throughout the tropical world. Detailed knowledge of the specific size and charge characteristics of the proteins that leak might also allow informed and rational decisions to be made with regard to the suitability of different fluid preparations for

Table 3. Urinary glycosaminoglycan:creatinine excretion ratios for children with acute dengue shock syndrome (DSS) vs. healthy control subjects.

<table>
<thead>
<tr>
<th>Group</th>
<th>Heparan sulfate, mg/mmol creatinine</th>
<th>Chondroitin-4-sulfate, mg/mmol creatinine</th>
<th>Chondroitin-6-sulfate, mg/mmol creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute DSS</td>
<td>0.47* (0.19–0.78)</td>
<td>0.37 (0.12–0.67)</td>
<td>0.54 (0.21–0.78)</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>0.23 (0.06–0.52)</td>
<td>0.31 (0.12–0.46)</td>
<td>0.48 (0.31–2.02)</td>
</tr>
</tbody>
</table>

* P = .016 vs. control group (Mann-Whitney U test).

NOTE. Data are median (90% range).

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the management of shock. At present, volume overload, which may be exacerbated by the leakage of resuscitation fluids, constitutes one of the major complications of DSS that contributes to mortality. Finally, if the molecular mechanisms responsible for the increased vascular permeability were better understood, it might be possible to design precise pharmacological interventions to counteract or prevent leakage.

Acknowledgments

We are grateful to all of the participants who participated in the study and their families. In Vietnam, we thank the doctors and nurses of the Paediatric Intensive Care Unit and the laboratory staff of the Oxford University Clinical Research Unit, in particular Pham Thi Doan. We also thank Kasia Stepniewska for statistical advice and Pat Kyd and the staff of the Chemical Pathology Department, St. Mary’s Hospital, London, for help with the laboratory analyses.

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